MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIATIONS IN *CLAVICEPS FUSIFORMIS* LOV.

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SUMMARY. – Variability in the pearl millet (*Pennisetum americanum* (L.) Leeke) ergot pathogen (*Claviceps fusiformis* Lov.) was studied in 7 isolates collected from distant pearl millet growing areas of Rajasthan State. There was no significant difference in morphology (size and shape) of conidia. However, conidia of all the isolates produced in culture were smaller than those produced on the host. When grown on seven synthetic media, the isolates could not be differentiated on the bases of colour and topography of cultures but differed significantly for growth (size of colony). Isolates x media interactions were significant for growth but not for sporulation. Inoculation of a susceptible pearl millet hybrid (BJ 104) with the isolates revealed that isolates differed significantly for incubation period, incidence, and number and weight of sclerotia. The SKR isolate proved to be most aggressive amongst the seven isolates. Inoculation with mixture of all the isolates (in equal proportions) gave intermediate reactions.

RÉSUMÉ. – La variabilité de l'ergot (*Claviceos fusiformis* Lov.) du mil (*Pennisetum americanum* (L.) Leeke) est étudiée pour 7 isolats récoltés dans différents champs de mil de l'État du Rajasthan. Aucune différence significative n'est relevée au niveau de la morphologie des conidies (taille et forme). Cependant, les conidies de tous les isolats produites en culture sont plus petites que celles produites sur l'hôte. Ensemencés sur 7 milieux synthétiques, les isolats ne peuvent pas être distingués par la couleur ou l'aspect des cultures, mais ils diffèrent significativement par la croissance (taille des colonies). Les interactions Isolat x Milieu sont significatives pour la croissance mais pas pour la sporulation. L'inoculation d'un mil hybride (BJ 104) révèle que les isolats diffèrent par la période d'incubation, l'aggressivité, le nombre et le poids des sclérotes obtenus. L'isolat SKR est le plus agressif et l'inoculation par un mélange de tous les isolats (en proportions identiques) donne une réaction intermédiaire.

KEY WORDS : Pearl millet, *Pennisetum americanum*, ergot, *Claviceps fusiformis*, variability.

Ergot (*Claviceps fusiformis* Lov.) of pearl millet (*Pennisetum americanum* (L.) Leeke) occurs widely in India (RAMASWAMY, 1968; SIDDIQUI & KHAN, 1973; VERMA & PATHAK, 1984) and African countries (RAMAKRISHNAN,

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Source : MNHN, Paris

1963; LOVELESS, 1967). The disease is one of the principal factors preventing the realization of high yield potentials from pearl millet hybrids in Asia and Africa (THAKUR & al., 1982). In addition, the cases of human and animal health hazards have been noticed due to consumption of pearl millet seeds contaminated by alkaloid-containing sclerotia (PATEL & al., 1958; SHONE & al., 1959; KRISHNAMACHARI & BHAT, 1976).

A number of grasses including different species of *Pennisetum* have been tested for locating sources of resistance and/or for their possible role as alternative/collateral hosts (RAMAKRISHNAN, 1952; SHINDE & BHIDE, 1958; VERMA, 1983). But the results obtained by these workers are highly contradictory which might be due to, in addition to other reasons, variations in the pathogen isolates. Since no disease management strategy can be perfect without understanding variability in pathogen, the present investigation was aimed to findout morphological, cultural and pathogenic variations in *C. fusiformis*.

MATERIALS AND METHODS

– **Preparation of isolates** : Ergoted earheads of pearl millet were collected from 7 distant areas of Rajasthan state. Sclerotia were harvested separately and the fungus was isolated, purified and single-spore culture of each isolate was maintained on Kirchoff's medium (KIRCHOFF, 1929). The isolates were named as BSL, JP, JOB, MRT, RNW, PHL and SKR. To maintain the isolates in honeydew stage, earheads of pearl millet hybrid BJ 104 (highly susceptible) raised under controlled conditions in a polythene house were separately inoculated by each isolate.

– **Morphology of conidia produced in culture** : Fifteen-day-old culture of each isolate in Petri dishes held at 25 ± 1°C was used to find out difference in conidial morphology. The conidia were taken from points equidistant from original inoculum. Hundred conidia of each isolate were measured.

– **Morphology of conidia produced on host** : Conidia were obtained from honeydew state of the pathogen maintained on earheads of pearl millet hybrid BJ 104 in polythene house. For each isolate, a drop of 3-day-old honeydew from each of 5 earheads were taken, mixed with sterile water and shaken thoroughly. A loopful of suspension was kept on each microscopic slide. For each isolate 100 conidia were measured.

– **Cultural variations** : Variations in growth and sporulation amongst the isolates were recorded by growing them on seven agar media viz., Asthana and Hawker's, Brown's, Czapek's, Dextrose-Asparagine-Phosphate and Elliott's media (PATHAK, 1972), Kirchoff's medium (KIRCHOFF, 1929) and modified Kirchoff's medium (NAGARAJAN & SARASWATHI, 1975). Each Petri dish containing 20 ml medium (pH 7.0) was inoculated in the centre with a 2 mm disc cut away from the periphery of a 15-day-culture (at 25°C) of the respective

Source : MNHN, Paris

isolate on Kirchoff's agar. The amount of growth was recorded by measuring radial growth along 2 diameters at right angle to each other. To record sporulation, a 2 mm disc was cut out from r/2 (distance between point of inoculation and periphery of radial growth - r) from each of the four replicates. Each disc was shaken in 10 ml of sterile water contained in 100 ml conical flasks. The shaking was done on a horizontal mechanical shaker for 10 minutes. Four drops of suspension from each flask were assessed for number of conidia with the help of haemocytometer.

– **Pathogenic variations** : Hot water-treated seeds of pearl millet hybrid BJ 104 were sown in polythene house. At boot stage, earheads of the plants were bagged in pollination bags and allowed to develop within the bags. At stigma bifid stage the bags were removed and individual earheads were inoculated by dipping into inoculum suspension (6.5×10^4 conidia/ml) for 30 seconds followed by rebagging. Besides the 7 spore suspensions representing the 7 isolates, a suspension of mixed isolates was also prepared by mixing suspensions of individual isolates in equal proportions. Each treatment was replicated thrice with 10 earheads in each replication. Following parameters were used to detect variations in the pathogenicity of the isolates.

Incubation period : Recorded as number of days taken for appearance of the first symptom in the inoculated earheads.

Incidence : Computed as $\frac{\text{No. of earheads infected}}{\text{No. of earheads inoculated}} \times 100$

Severity : Recorded as percentage of earhead area infected using a disease assessment key. Each earhead was individually assessed for the per cent area affected on the two sides.

Number of sclerotia : The total sclerotia formed in the earheads of each replication were counted and overall number/earhead was calculated.

Weight (size) of 100 sclerotia : Due to highly irregular shape it was difficult to measure size of sclerotia. The size of sclerotia was therefore expressed as weight of sclerotia. Because a separate experiment revealed that the size (volume) of sclerotia was positively correlated with their weight. To record weight, sclerotia threshed from the earheads of each replication were mixed, washed thoroughly with water and dried in sun. From these, 100 sclerotia were taken randomly and weight.

OBSERVATIONS

– **Morphological variations** : There were no significant differences in the size of conidia of the seven isolates. Conidia of the pathogen in culture were smaller (6.22-15.28 x 1.91-3.82 μm) than those formed in earheads (9.55-21.96 x 2.86-4.77 μm). Conidia of all the isolates were broadly falcate, fusiform, hyaline and single-celled.

Source : MNHN, Paris

— **Cultural variations** : The isolates could not be differentiated on the bases of colour and topography of cultures but differed in respect of size of colony. Growth of MRT isolate was maximum (39.67 mm) and it was significantly more than remaining isolates. On comparing the overall means for media, it was found that growth on different media differed significantly from each other. Modified Kirchoff's medium produced maximum growth (50.50 mm). Media x isolates interactions were also significant indicating differential behaviour of isolates on different media.

Sporulation was maximum (8.92 x 10^5 conidia/ml) in JOB isolate and minimum (4.82 x 10^5 conidia/ml) in BSL isolate. On comparing the over all means for media, it was revealed that Kirchoff's medium supported maximum 14.28 x 10^5 conidia/ml) and significantly more sporulation. Unlike growth, isolates x media interactions were nonsignificant for sporulation.

— **Pathogenic variations** : The isolates differed significantly for incubation period, incidence and number and weight of sclerotia, but not for severity (Table 1). Incubation period, number of sclerotia and weight of sclerotia were rather more powerful parameters in discriminating the isolates. SKR isolate was proved to be most aggressive amongst all the isolates. Inoculation with mixture of the seven isolates gave intermediate reactions.

Isolate	Parameters				
	Incubation period (days)	Incidence (%)	Severity (%)	Sclerotia (number)	Weight of 100 sclerotia (g)
BSL	4.80	73.33 (59.01)	26.16 (30.68)	38.87	0.993
JOB	4.67	80.00 (67.86)	29.65 (32.75)	89.90	1.093
JP	5.13	83.33 (66.15)	22.61 (28.39)	26.73	1.017
MRT	5.07	70.00 (57.00)	21.08 (27.19)	24.80	0.767
PHL	4.63	76.66 (61.22)	23.13 (26.06)	41.93	1.230
RNW	5.13	73.33 (59.01)	27.83 (31.80)	55.97	0.757
SKR	4.30	100.00 (90.00)	33.66 (35.41)	93.33	1.420
MIX	4.70	76.66 (61.22)	28.70 (32.34)	38.23	0.608
$LSD_{(5\%)}$	0.47	13.54	NS	4.69	0.123

Figures in parenthesis are angular values.

Table 1. — Pathogenic variability amongst the isolates of *C. fusiformis*.
Tableau 1. — Variations dans la pathogénie d'isolats de *C. fusiformis*.

DISCUSSION

Morphologically, conidia of all the seven isolates were similar. FRAUENSTEIN (1972) after studying conidia of *C. purpurea* from 17 different hosts

Source : MNHN, Paris

also concluded that conidial shape did not depend on fungus but on host and warned that conidial shape and size are unsuitable for identification of physiologic forms. The fact that media x isolates interactions were significant only for growth suggests that isolates were more sensitive to constituents of media for growth than for sporulation.

Detection of pathogenic variability within seven isolates only, is an indication of extreme pathogenic variability in the pearl millet ergot pathogen. Due considerations must therefore be given to pathogenic variations in resistance screening programmes. Otherwise it is likely that such attempts may not be fruitful or the located sources of resistance would not be long lasting. Variability in isolates with respect to number of sclerotia, size of sclerotia and honeydew production has also been reported in *C. purpurea* (PLATFORD & BERNIER, 1976). The suggestion of DARLINGTON & al. (1977) for using the mixture of isolates for host resistance screening against *C. purpurea* due to variation in virulence of isolates may hold good for *C. fusiformis* also.

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